



**INSTITUTE OF INDUSTRIAL ORGANIC CHEMISTRY
ANALYTICAL DEPARTMENT**

ANNOPOL 6, 03-236 WARSAW, POLAND

Phone: +48 22 811 12 31

Fax: +48 22 811 07 99

e-mail: wzorce@ipo.waw.pl

www.ipo.waw.pl



**CERTIFICATE OF ANALYSIS
REFERENCE MATERIAL No. IPO 505**

NICOTINE

(S)-3-(1-methylpyrrolidin-2-yl)pyridine

C₁₀H₁₄N₂ (162.2)

CAS REG. No. [54-11-5]

Series No. 1K/15

Valid to: June 2019

Purity: 99.6 ± 0.2 % (m/m)

Unit: 0.5 g of liquid in a brown glass ampoule sealed under argon.

Storage: The material should be stored in the original closed vial in a refrigerator at 5 ± 4 °C until it is required to use. Prolonged storage approx -20 °C. Allow to equilibrate to ambient temperature before opening. It is intended for use as a reference material for the calibration of measuring equipment, for the evaluation of analytical procedures and for the study of biological activity.

CONFIRMATION OF THE IDENTITY

The identity of the product was established by infrared spectroscopy and mass spectrometry.

The IR spectrum (film, scanning from 4000 to 400 cm⁻¹) of sample was compared with the literature spectrum^{2,3}. The spectrum of the sample was consistent with the spectral libraries for Nicotine. The mass spectrum of sample was also recorded by GC/MS (column DB-5 MS (30 m x 0.25 mm x 0.25 μm), temperature program 50 °C (3 min) to 140 °C (5 °C/min); 140 °C (5 min) to 240 °C (10 °C/min), ion source temp. 250 °C, injector 240 °C EI, 70 eV,) and the molecular ion was obtained M⁺ m/z 162 and no differences were observed in comparison with the literature spectrum^{1,2,3}.

DETERMINATION OF THE PURITY

Representative samples were drawn from the bulk material. The purity value was based on determinations made on these representative samples using the following techniques:

- gravimetry
- high performance liquid chromatography (HPLC)
- gas chromatography (GC)
- determination of water content (KF)

The uncertainties quoted below are the half-width of a 95 % confidence interval based on the standard deviation of the results obtained. The certified uncertainty is the combined uncertainty calculated according to the methodology described⁴ with a coverage factor k = 2. It corresponds to a confidence level of 95 %.

Gravimetry

The method used for the gravimetric determination of nicotine was ISO 13276:1997 -Tobacco and tobacco products-Determination of nicotine purity-Gravimetric technique using tungstosilicic acid.

Result: 99.83 ± 0.23 % (m/m)(n = 4).

Determination by GC

Column I: fused-silica DB-1 (30 m x 0.53 mm i.d.), film thickness 3.0 µm.

Temperature conditions:

- Column: 40 °C (5 min) to 140 °C (5 °C/min), 140 °C (10 min) to 240°C (5 °C/min)
- Injector: 200 °C
- Detector (flame ionisation): 270 °C

Carrier gas: 6.9 ml/min.

Injection volume: 0.2 µl of sample solutions in acetone (0.4-1.3 % *m/V*).

The purity was calculated by peak area normalisation method.

Result: six impurities were detected with a total concentration of 0.33 ± 0.08 % (*m/m*) (n = 4).

Column II: fused-silica HP-1701 (30 m x 0.53 mm i.d.), film thickness 1.0 µm.

Temperature conditions:

- Column: 40 °C (3 min) to 140 °C (5 °C/min), 140 °C (10 min) to 230 °C (5 °C/min)
- Injector: 200 °C
- Detector (flame ionisation): 270 °C

Carrier gas: 9.0 ml/min.

Injection volume: 0.2 µl of sample solutions in acetone (0.4-1.3 % *m/V*).

The purity was calculated by peak area normalisation method.

Result: seven impurities were detected with a total concentration of 0.37 ± 0.04 % (*m/m*) (n = 4)

Determination by HPLC

- Column: Kinetex Biphenyl C₁₈, 5 µm, 250 x 4.6 mm i.d.
- Mobile phase: phosphate buffer (0.05M, pH 5.0)+acetonitrile + metanol +THF (92+5+2+1) + 0.2 % triethylamine
- Flow rate: 0.5 ml/min
- UV detection at 260 nm.
- Injection volume: 20 µl of sample solutions in mobile phase (0.05 - 0.07 % *m/V*).

The purity was determined assuming equal detector response factors for all constituents.

Three impurities were detected with a total concentration of 0.08 ± 0.01 % (*m/m*) (n = 5).

Determination of water content

By the Karl Fischer technique. Result: 0.11 % (*m/m*) (n=2).

CONCLUSIONS

On the basis of the results of three independent quantitative techniques (gravimetry, GC and HPLC) and other determinations, the purity of this batch of Nicotine was assessed as 99.6 ± 0.2 % (*m/m*).

The analytical measurements were coordinated by:



Anna Kielczewska, MSc

Head of Analytical Department



Maciej Śliwakowski, PhD

October 2015

Distribution: LGC Standards GmbH, Mercatorstr. 51, 46485 Wesel, Germany

Tel: +49 (0)281/9887 140

Fax: +49 (0)281/9887 149

e-mail: de@lgcstandards.com

www.lgcstandards.com

1. Database NIST MS Serach v. 2.0, ID # 90290
2. Integrated Spectral Data Base System for Organic Compounds, SDDBS No. 6729, National Institute of Advanced Industrial Science and Technology, Tsukuba, Ibaraki, Japan, www.aist.go.jp/RIODB/SDDBS
3. MS, NMR, IR, UV Atlas, Spectra Collection of Pesticides, Drugs and Pollutants, Spectral Service GmbH, Köln, Riedel-de Haën AG, Seelze, Germany, Vol. P3 # 36733 (1992).
4. NIST Special Publication 1012. An approach to the metrologically sound traceable assessment of the chemicals purity of organic reference materials David L. Duever and others, US Department of Commerce USA, September 2004, p.22